WHAT IS CLAIMED IS:

1. A wafer level microfabricated biosensor comprising:

(a) a base sensor;

(b) a permselective layer, superimposed over at least a portion of said base sensor, having a thickness sufficient to exclude substantially molecules with a molecular weight of about 120 or more while allowing the free permeation of molecules with a molecular weight of about 50 or less; an

(c) a biolayer superimposed over at least a portion of said permselective layer, comprising (i) a sufficient amount of a bioactive molecule capable of selectively interacting with a particular analyte species, and (ii) a support matrix in which said bioactive molecule is incorporated, which matrix is derived from the group consisting of a photoformable proteinaceous mixture, a film-forming latex, and combinations thereof and through which matrix said analyte species may freely permeate and interact with said bioactive molecule.

2. The microfabricated biosensor of claim 1 in which said permselective layer comprises a polymer film.

3. The microfabricated biosensor of claim 1 in which said permselective layer comprises a heat-treated film of a silane compound having the formula $R'_nSi(OR)_{4-n}$, in which n is an integer selected from the group consisting of 0, 1, and 2; R' is a hydrocarbon radical comprising 3-12 carbon atoms; and R is a hydrogen radical or a lower alkyl radical comprising 1-4 carbon atoms.

4. The microfabricated biosensor of claim 1 which further comprises an electrolyte layer interposed between said base sensor and said permselective layer.

5. The microfabricated biosensor of claim 4 in which said permselective layer substantially encloses said electrolyte layer.

6. The microfabricated biosensor of claim 1 which further comprises an analyte attenuation layer, superimposed over a substantial portion of said biolayer, having a thickness sufficient to attenuate the transport therethrough of analyte species with a molecular weight of about 120 or more.

7. The microfabricated biosensor of claim 6 in which said analyte attenuation layer comprises a polymer film.

8. The microfabricated biosensor of claim 6 which further comprises a photoresist cap superimposed over said analyte attentuation layer.

9. The microfabricated biosensor of claim 2 in which a sufficient amount of an ionophore is incorporated in said polymer film.

10. The microfabricated biosensor of claim 1 in which said base sensor comprises an electrochemical transducer.

11. The microfabricated biosensor of claim 10 in which said electrochemical transducer is amperometric.

12. The microfabricated biosensor of claim 10 in which said electrochemical transducer is potentiometric.

13. The microfabricated biosensor of claim 10 in which said electrochemical transducer comprises a noble late transition metal electrode.

14. The microfabricated biosensor of claim 1 in which said base sensor comprises an amperometric electrochemical transducer comprising an indicator electrode which includes an electrocatalyst selected from the group consisting of carbon, platinum, gold, silver, rhodium, iridium, ruthenium, mercury, palladium, and osmium.

15. The microfabricated biosensor of claim 13 in which said electrochemical transducer further comprises a reference electrode which includes an electrocatalyst metal selected from the group consisting of silver, gold, and platinum.

16. The microfabricated biosensor of claim 13 in which said electrochemical transducer further comprises a silver/silver halide reference electrode.

17. The microfabricated biosensor of claim 3 in which said silane compound is selected from the group consisting of 3-aminopropyltriethoxysilane, N-(2-aminoethyl)-3-aminopropyltriethoxysilane, 3-aminopropyltrimethoxysilane, N-(2-aminoethyl)-3-aminopropyltrimethoxysilane, 3-isocyanatopropyltriethoxysilane,10-aminodecyltrimethoxysilane, 11-aminoundecyltrimethoxysilane, 2-[p-(N-(2-aminoethyl)aminomethyl)phenyl]ethyltrimethoxysilane, n-propyltrimethoxysilane, phenyltrimethoxysilane, diethylphosphatoethyltriethoxysilane, N,N-bis(2-hydroxyethyl)aminopropyltriethoxysilane, 3-chloropropyltriethoxysilane, and mixtures thereof.

18. The microfabricated biosensor of claim 3 in which said silane compound is selected from the group consisting of tetramethyl orthosilicate, tetraethyl orthosilicate, tetrapropyl orthosilicate, tetrabutyl orthosilicate, and mixtures thereof.

19. The microfabricated biosensor of claim 2 or 7 in which said polymer film comprises a polymeric substance selected from the group consisting of polyurethane, poly(vinyl chloride), poly(tetrafluoroethylene), cellulose acetate, cellulose nitrate, silicone rubber, derivatives, and mixtures thereof.

20. The microfabricated biosensor of claim 9 in which said ionophore is selected from the group consisting of crown ethers, trialkylamines, phosphate esters, valinomycin, nonactin, monensin, methylmonensin, and mixtures of monensin and methylmonensin.

21. The microfabricated biosensor of claim 9 in which said ionophore is a quaternary ammonium halide.

22. The microfabricated biosensor of claim 2 or 7 in which said polymer film comprises a copolymer of a siloxane compound and a nonsiloxane compound.

23. The microfabricated biosensor of claim 22 in which said copolymer is selected from the group consisting of dimethylsiloxane-alkene oxide, tetramethyldisiloxane-divinylbenzene, tetramethyldisiloxane-ethylene, dimethylsiloxane-silphenylene, dimethylsiloxane-silphenylene oxide, dimethylsiloxane-methylstyrene, and dimethylsiloxane-bisphenol A carbonate, and mixtures thereof.

24. The microfabricated biosensor of claim 22 in which said copolymer is dimethylsiloxane-bisphenol A carbonate.

25. The microfabricated biosensor of claim 1 in which said photoformable proteinaceous mixture comprises: (i) a proteinaceous substance; (ii) an effective amount of a photosensitizer uniformly dispersed in said proteinaceous substance; and (iii) water.

26. The microfabricated biosensor of claim 25 in which said proteinaceous substance is selected from the group consisting of albumin, casein, gamma-globulin, collagen, derivatives, and mixtures thereof.

27. The microfabricated biosensor of claim 25 in which said proteinaceous substance is fish gelatin.

28. The microfabricated biosensor of claim 25 in which said photosensitizer is selected from the group consisting of ferric chloride, ferric ammonium citrate, ferric potassium citrate, ~~ferric ammonium~~ ferric ammonium oxalate, ferric sodium oxalate, ferric potassium oxalate, ferric oxalate, potassium dichromate, and ammonium dichromate.

29. The microfabricated biosensor of claim 25 in which said photoformable proteinaceous mixture further comprises a porosity-altering substance selected from the group consisting of polyhydroxylated compounds, salts, and mixtures thereof.

30. The microfabricated biosensor of claim 1 in which said film-forming latex comprises an aqueous emulsion of a polymer or copolymer derived from synthetic or natural sources.

31. The microfabricated biosensor of claim 1 in which said film-forming latex further comprises a porosity-altering substance selected from the group consisting of polyhydroxylated compounds, salts, and mixtures thereof.

32. The microfabricated biosensor of claim 1 in which said film-forming latex further comprises a crosslinking agent.

33. A wafer level microfabricated biosensor comprising:

(a) a base sensor;

(b) a permselective layer, superimposed over at least a portion of said base sensor, having a thickness sufficient to exclude substantially molecules with a molecular weight of about 120 or more while allowing the free permeation of molecules with a molecular weight of about 50 or less; and

(c) a biolayer superimposed over at least a portion of said permselective layer and said base sensor, comprising (i) a sufficient amount of a bioactive molecule capable of selectively interacting with a particular analyte species, and (ii) a support matrix derived from a photoformable proteinaceous mixture, in which said bioactive molecule is incorporated, and through which matrix said analyte species may freely permeate and interact with said bioactive molecule.

34. A wafer level microfabricated biosensor comprising:

(a) a base sensor;

(b) a permselective layer, superimposed over at least a portion of said base sensor, having a thickness sufficient to exclude substantially molecules with a molecular weight of about 120 or more while allowing the free permeation of molecules with a molecular weight of about 50 or less; and

(c) a biolayer superimposed over at least a portion of said permselective layer and said base sensor, comprising (i) a sufficient amount of a bioactive molecule capable of selectively interacting with a particular analyte species, and (ii) a support matrix derived from a film-forming latex, in which said bioactive molecule is incorporated, and through which matrix said analyte species may freely permeate and interact with said bioactive molecule.

35. The microfabricated biosensor of claim 1, 4, 6, 33, or 34 in which said bioactive molecule is an enzyme selected from the group consisting of glucose oxidase, glucose dehydrogenase, NADH oxidase, uricase, urease, creatininase, sarcosine oxidase, creatinase, creatine kinase, creatine amidohydrolase, cholesterol esterase, cholesterol oxidase, glycerol kinase, hexokinase, glycerol-3-phosphate oxidase, lactate dehydrogenase, alkaline phosphatase, alanine transaminase, aspartate transaminase, amylase, lipase, esterase, gamma-glutamyl transpeptidase, L-glutamate oxidase, pyruvate oxidase, diaphorase, bilirubin oxidase, and their mixtures.

36. The microfabricated biosensor of claim 1, 4, 6, 33, or 34 in which said bioactive molecule is selected from the group consisting of ionophores, cofactors, polypeptides, proteins, glycoproteins, enzymes, immunoglobulins, antibodies, antigens, lectins, neurochemical receptors, oligonucleotides, polynucleotides, molecules of DNA, molecules of RNA, active fragments or subunits or single strands of the preceding molecules, and mixtures thereof.

37. The microfabricated biosensor of claim 1, 4, 6, 33, or 34 in which said bioactive molecule is glucose oxidase.

38. The microfabricated biosensor of claim 1, 4, 6, 33, or 34 in which said bioactive molecule is urease.

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39. A microfabricated biosensor comprising:

(a) a base sensor;

(b) a permselective layer, superimposed over at least a portion of said base sensor, having a thickness sufficient to exclude substantially molecules with a molecular weight of about 120 or more while allowing the free permeation of molecules with a molecular weight of about 50 or less; and

(c) a topmost layer comprising a sufficient amount of an immobilized ligand receptor.

40. The microfabricated biosensor of claim 39 in which said permselective layer comprises a polymer film with available reactive functional groups on its outer surface.

41. The microfabricated biosensor of claim 39 in which said permselective layer comprises a heat-treated film of a silane compound having the formula $R'_nSi(OR)_{4-n'}$ in which n is an integer with a value of 1 or 2; R' is a hydrocarbon radical, comprising 3-12 carbon atoms, having a terminal reactive functional group; and R is a hydrogen radical or a lower alkyl radical comprising 1-4 carbon atoms.

42. A microfabricated biosensor comprising:

(a) a base sensor;

(b) an adhesion promoting layer, localized over preselected areas of said base sensor, which layer comprises a film of a silane compound having the formula $R'_nSi(OR)_{4-n'}$ in which n is an integer with a value of 1 or 2; R' is a hydrocarbon radical, comprising 3-12 carbon atoms, having a

terminal reactive functional group; and R is a hydrogen radical or a lower alkyl radical comprising 1-4 carbon atoms; and

(c) a topmost layer comprising a sufficient amount of an immobilized ligand receptor.

41 wafer level
~~43~~. A microfabricated biosensor comprising:

(a) a base sensor;

(b) a permselective layer comprising a polymer film, superimposed over at least a portion of said base sensor and having a thickness sufficient to exclude substantially molecules with a molecular weight of about 120 or more while allowing the free permeation of molecules with a molecular weight of about 50 or less;

(c) a photoresist layer comprising a photoformable proteinaceous mixture, superimposed over a substantial portion of said permselective layer; and

(d) a topmost layer comprising a sufficient amount of an immobilized ligand receptor.

42 41
~~44~~. The microfabricated biosensor of claim ~~43~~ which further comprises an electrolyte layer interposed between said base sensor and said permselective layer.

43 41
~~45~~. The microfabricated biosensor of claim 39, ~~42~~ or ~~43~~ which said ligand receptor is selected from the group consisting of ionophores, cofactors, polypeptides, proteins, glycoproteins, enzymes, immunoglobulins, antibodies, antigens, lectins, neurochemical receptors, oligonucleotides, polynucleotides, molecules of DNA, molecules of RNA, active fragments or subunits or single strands of the preceding molecules, and mixtures thereof.

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~~46.~~ A wafer comprising:

(a) a substantially planar substrate; and

(b) an array of unit cells having uniform dimensions established on said substrate, each unit cell comprising a microfabricated biosensor of claim 1, 4, 6, 33, 34, 39, ~~42,~~ or 41 ~~43~~.

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~~47.~~ A biolayer sensitive to a particular analyte species comprising:

(a) a sufficient amount of a bioactive molecule capable of selectively interacting with a particular analyte species; and

(b) a support matrix in which said bioactive molecule is incorporated, which matrix is derived from the group consisting of a photoformable proteinaceous mixture, a film-forming latex, and combinations thereof and through which said analyte species may freely permeate and interact with said bioactive molecule.

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~~48.~~ A solid object having an outer surface, an inner surface, or both, over at least a portion of which surface is established a biolayer sensitive to a particular analyte species comprising: (i) a sufficient amount of a bioactive molecule capable of selectively interacting with a particular analyte species; and (ii) a support matrix in which said bioactive molecule is incorporated, which matrix is derived from the group consisting of a photoformable proteinaceous mixture, a film-forming latex, and combinations thereof and through which said analyte species may freely permeate and interact with said bioactive molecule.

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~~49.~~ The solid object of claim 46 ~~48~~ which comprises part of a diagnostic system.

48 ~~50.~~ The solid object of claim 46 ~~48~~ which comprises part of a bioreactor.

49 ~~51.~~ A permselective layer comprising a heat-treated film of a silane compound having a formula $R'_nSi(OR)_{4-n}$ in which n is an integer selected from the group consisting of 0, 1, and 2; R' is a hydrocarbon radical comprising 3-12 carbon atoms; and R is a lower alkyl radical comprising 1-4 carbon atoms,

said layer having a thickness sufficient to exclude substantially molecules with a molecular weight of about 120 or more while allowing the free permeation of molecules with a molecular weight of about 50 or less.

50 ~~52.~~ An analyte attenuation layer comprising a film of a siloxane-nonsiloxane copolymer and which film has a thickness sufficient to attenuate the transport therethrough of analyte species having a molecular weight of about 120 or more.

51 ~~53.~~ The analyte attenuation layer of claim 50 ~~52~~ ~~52in~~ in 1 which said copolymer is selected from the group consisting of dimethylsiloxane-alkene oxide, tetramethyldisiloxane-divinylbenzene, tetramethyldisiloxane-ethylene, dimethylsiloxane-silphenylene, dimethylsiloxane-silphenylene oxide, dimethylsiloxane-methylstyrene, dimethylsiloxane-bisphenol A carbonate, and mixtures thereof.

54. A method of manufacturing a plurality of uniform microfabricated sensing devices which comprises:

(a) establishing a plurality of base sensors on a suitable substrate wafer;

(b) establishing a permselective layer, superimposed over at least a portion of each base sensor, having a thickness sufficient to exclude substantially

molecules with a molecular weight of about 120 or more while allowing the free permeation of molecules with a molecular weight of about 50 or less; and

(c) establishing a support matrix, superimposed over at least a portion of said permselective layer and each of said base sensors, which matrix is derived from the group consisting of a photoformable proteinaceous mixture, a film-forming latex, and combinations thereof, and which is capable of incorporating a bioactive molecule which, in turn, is capable of selectively interacting with a particular analyte species,

to form a plurality of uniform microfabricated sensing devices.

55. The method of claim 54 which further comprises contacting said matrix with a sufficient amount of said bioactive molecule.

56. A method of manufacturing a plurality of uniform microfabricated sensing devices which comprises:

(a) establishing a plurality of base sensors on a suitable substrate wafer;

(b) establishing a permselective layer superimposed over at least a portion of each base sensor; and

(c) establishing a biolayer, superimposed over at least a portion of said permselective layer and each of said base sensors, said biolayer comprising (i) a sufficient amount of a bioactive molecule, and (ii) a support matrix in which said bioactive molecule is incorporated, which matrix is derived from the group consisting of a photoformable proteinaceous mixture, a film-forming latex, and combinations thereof to form a plurality of uniform microfabricated sensing devices.

57. A method of manufacturing a plurality of uniform microfabricated sensing devices which comprises:

(a) establishing a plurality of base sensors on a suitable substrate wafer;

(b) establishing a permselective layer, superimposed over at least a portion of each base sensor, having a thickness sufficient to exclude substantially molecules with a molecular weight of about 120 or more while allowing the free permeation of molecules with a molecular weight of about 50 or less; and

(c) establishing a biolayer, superimposed over at least a portion of said permselective layer and each of said base sensors, said biolayer comprising (i) a sufficient amount of a bioactive molecule, and (ii) a support matrix, in which said bioactive molecule is incorporated, which matrix is derived from a photoformable proteinaceous mixture,

to form a plurality of uniform microfabricated sensing devices.

58. A method of manufacturing a plurality of uniform microfabricated sensing devices which comprises:

(a) establishing a plurality of base sensors on a suitable substrate wafer;

(b) establishing a permselective layer, superimposed over at least a portion of each base sensor, having a thickness sufficient to exclude substantially molecules with a molecular weight of about 120 or more while allowing the free permeation of molecules with a molecular weight of about 50 or less; and

(c) establishing a biolayer, superimposed over at least a portion of said permselective layer and each of said base sensors, said biolayer comprising (i) a sufficient amount of a bioactive molecule, and (ii) a support matrix, in which said bioactive molecule is incorporated, which matrix is derived from a film-forming latex,

to form a plurality of uniform microfabricated sensing devices.

59. A method of manufacturing a plurality of uniform microfabricated sensing devices which comprises:

(a) establishing a plurality of base sensors on a suitable substrate wafer;

(b) establishing a permselective layer, superimposed over at least a portion of each base sensor, having a thickness sufficient to exclude substantially molecules with a molecular weight of about 120 or more while allowing the free permeation of molecules with a molecular weight of about 50 or less; and

(c) establishing a topmost layer comprising a sufficient amount of an immobilized ligand receptor.

60. The method of claim 59 in which said permselective layer comprises a polymer film with available reactive functional groups on its outer surface.

61. A method of manufacturing a plurality of uniform microfabricated sensing devices which comprises:

(a) establishing a plurality of base sensors on a suitable substrate wafer;

(b) establishing an adhesion promoting layer, localized over preselected areas of said base sensor, which layer comprises a film of a silane compound having the formula $R'_nSi(OR)_{4-n}$, in which n is an integer with a value of 1 or 2; R' is a hydrocarbon radical, comprising 3-12 carbon atoms, having a terminal reactive functional group; and R is a hydrogen radical or a lower alkyl radical comprising 1-4 carbon atoms; and

(c) establishing a topmost layer comprising a sufficient amount of an immobilized ligand receptor.

62. A method of manufacturing a plurality of uniform microfabricated sensing devices which comprises:

(a) establishing a plurality of base sensors on a suitable substrate wafer;

(b) establishing a permselective layer, superimposed over at least a portion of each base sensor, having a thickness sufficient to exclude substantially molecules with a molecular weight of about 120 or more while allowing the free permeation of molecules with a molecular weight of about 50 or less;

(c) establishing a photoresist layer comprising a photoformable proteinaceous mixture, superimposed over a substantial portion of said permselective layer; and

(d) establishing a topmost layer comprising a sufficient amount of an immobilized ligand receptor.

63. The method of claim 62 which further comprises establishing an electrolyte layer over at least a portion of each base sensor, prior to establishing said permselective layer.

64. The method of claim 59, 61 or 62 in which said ligand receptor is an immunoreactive species.

65. A method of forming a permselective layer which comprises:

(a) establishing at least one film comprising a silane compound mixed with a suitable solvent, said compound having the formula $R'_nSi(OR)_{4-n'}$ in which n is an integer selected from the group consisting of 0, 1, and 2; R' is a hydrocarbon radical comprising 3-12 carbon atoms; and R is a hydrogen radical or a lower alkyl radical comprising 1-4 carbon atoms; and

(b) heating said film to a temperature of at least about 100°C for a period of time effective to form a permselective layer, having a thickness sufficient to provide said permselective layer with the desired semipermeable properties.

66. The method of claim 65 in which said permselective layer is formed on a substantially planar sensing device.

67. A method for forming a permselective layer on preselected areas of a substantially planar sensing device which comprises:

(a) establishing a photoresist layer on a substantially planar sensing device;

(b) processing said photoresist layer to expose preselected areas of said sensing device;

(c) establishing at least one film comprising a silane compound mixed with a suitable solvent on the sensing device of step (b), said compound having the formula $R'_nSi(OR)_{4-n}$, in which n is an integer selected from the group consisting of 0, 1, and 2; R' is a hydrocarbon radical comprising 3-12 carbon atoms; and R is a hydrogen radical or a lower alkyl radical comprising 1-4 carbon atoms; and

(d) heating said film to a temperature of at least about 100°C for a period of time effective to form a permselective layer, having a thickness sufficient to provide said permselective layer with the desired semipermeable properties; and

(e) removing said photoresist layer and the overlaid permselective layer from all except the preselected areas of said sensing device.

68. A method of forming a permselective layer on preselected areas of a substantially planar sensing device which comprises:

(a) establishing at least one film comprising a silane compound mixed with a suitable solvent on the sensing device of step (b), said compound having the formula $R'_nSi(OR)_{4-n}$, in which n is an integer selected from the group consisting of 0, 1, and 2; R' is a hydrocarbon radical comprising 3-12 carbon atoms; and R is a hydrogen radical or a lower alkyl radical comprising 1-4 carbon atoms; and

(b) heating said film to a temperature of at least about 100°C for a period of time effective to form a permselective layer, having a thickness sufficient to provide said permselective layer with the desired semipermeable properties; and

(c) establishing a photoresist layer on said permselective layer;

(d) processing said photoresist layer such that a proportion of the underlying permselective layer becomes exposed and subject to further processing, while those preselected areas of the device retain a protective cap of photoresist material;

(e) removing said exposed permselective layer; and

(f) removing said protective photoresist layer to leave a permselective layer over preselected areas of the device.

69. The method of claim 66, 67 or 68 in which the thickness of said permselective layer is such that said permselective layer is permeable to molecules having a molecular weight of about 50 or less, yet substantially impermeable to molecules having a molecular weight of about 120 or more.

70. The method of claim 66, 67 or 68 in which said permselective layer is further characterized as: (i) having a thickness in the range of about 50 to about 100 Å; (ii) being

permeable to molecules selected from the group consisting of dioxygen and hydrogen peroxide; and (iii) being substantially impermeable to molecules selected from the group consisting of uric acid, ascorbic acid, salicylic acid, 2-(p-isobutylphenyl)propionic acid, cysteine, 4-acetamidophenol, and physiological salts thereof.

71. The method of claim 66, 67 or 68 in which said sensing device is an amperometric electrochemical sensor.

72. The method of claim 66, 67 or 68 in which said film is established by a means selected from the group consisting of spin-coating, dip-coating, spray-coating, and microdispensing.

73. A method of preventing interfering electroactive species from undergoing a redox reaction at the indicator electrode of an amperometric electrochemical sensor while permitting the free interaction of desired electroactive species with said sensor which comprises: (i) establishing a film comprising a silane compound mixed with a suitable solvent over an area which encompasses the indicator electrode of said electrochemical sensor, said compound having the formula $R'_nSi(OR)_{4-n}$, in which n is an integer selected from the group consisting of 0, 1, and 2; R' is a hydrocarbon radical comprising 3-12 carbon atoms; and R is a hydrogen radical or a lower alkyl radical comprising 1-4 carbon atoms; and (ii) heating said film to a temperature of at least about 100°C for a period of time effective to form a permselective layer

said permselective layer further characterized as having a thickness such that said permselective layer is permeable to molecules having a molecular weight of about 50 or less, yet substantially impermeable to molecules having a molecular weight of about 120 or more.

74. The method of claim 73 in which said permselective layer is further characterized as: (i) being permeable to dioxygen or hydrogen peroxide; and (ii) being substantially impermeable to uric acid, ascorbic acid, salicylic acid, cysteine, 4-acetamidophenol, or physiological salts thereof.

75. A method of detecting the presence and quantity of at least one analyte species in a liquid sample which comprises:

(a) contacting a microfabricated biosensor with a liquid sample, said biosensor comprising: (i) a base sensor, (ii) a permselective layer, superimposed over at least a portion of said base sensor, having a thickness sufficient to exclude substantially molecules with a molecular weight of about 120 or more while allowing the free permeation of molecules with a molecular weight of about 50 or less, and (iii) a biolayer superimposed over at least a portion of said permselective layer and said base sensor, which biolayer comprises a sufficient amount of a bioactive molecule capable of selectively interacting with a particular analyte species, and a support matrix in which said bioactive molecule is incorporated, which matrix is derived from the group consisting of a photoformable proteinaceous mixture, a film-forming latex, and combinations thereof and through which matrix said analyte species may freely permeate and interact with said bioactive molecule,

to obtain a measured signal output from which the presence and quantity of an analyte species may be deduced.

76. The method of claim 75 which further comprises contacting said biosensor with a suitable calibrant solution to obtain a reference signal output to which said measured signal output may be compared.

77. The method of claim 75 or 76 in which said liquid sample is a biological fluid.

78. The method of claim 75 or 76 in which said analyte species is selected fromthe group consisting of sodium ion, potassium ion, protons, chloride ion, ionized calcium, dissolved carbon dioxide, total carbon dioxide, dissolved oxygen, hydrogen peroxide, ethanol, glucose, cholesterol, uric acid, ascorbic acid, bilirubin, creatinine, creatine, triglyceride, lactate dehydrogenase, creatine kinase, alkaline phosphatase, creatine kinase-MB, alanine transaminase, aspartate transaminase, amylase, and lipase.

79. A method of detecting a plurality of analyte species in a single liquid sample which comprises (a) contacting said liquid sample with a calibrated wholly microfabricated biosensor comprising an array of overlaid structures each sensitive to a particular analyte species which structures are comprised of (i) a base sensor, (ii) a permselective layer, superimposed over at least a portion of said base sensor, having a thickness sufficient to exclude substantially molecules with a molecular weight of about 120 or more while allowing the free permeation of molecules with a molecular weight of about 50 or less, and (iii) a biolayer superimposed over at least a portion of said permseletive layer and said base sensor, which biolayer comprises a sufficient amount of a bioactive molecule capable of selectively interacting with a particular analyte species, and a support matrix in which said bioactive molecule is incorporated, which matrix is derived from the group consisting of a photoformable proteinaceous mixture, a film-forming latex, and combinations thereof and through which matrix said analyte species may freely permeate and interact with said bioactive molecule to obtain a plurality of signal

outputs from which the presence and quantity of each analyte species may be deduced; and (b) processing said signal outputs.

80. A method for assaying a sample for a particular ligand (analyte) species which comprises:

(a) providing reagents capable of interacting with a sample suspected of containing a particular ligand species to produce a change in the concentration of a detectable species, which change is proportional to the amount of said particular ligand species in said sample;

(b) contacting said sample and said reagents with a microfabricated biosensor comprising (i) a base sensor sensitive to the concentration of said detectable species; (ii) a permselective layer, superimposed over at least a portion of said base sensor, having a composition and thickness sufficient to exclude substantially molecules with a molecular weight of about 120 or more while allowing the free permeation of molecules with a molecular weight of about 50 or less; and (iii) a receptor layer, superimposed over said base sensor and at least a portion of said permselective layer, comprising a sufficient amount of an immobilized ligand receptor capable of binding said particular ligand species or any complex thereof;

(c) measuring the change in the concentration of said detectable species; and

(d) relating said change to the amount of said particular ligand species in said sample.

81. A method for assaying a sample for a particular ligand (analyte) species, which method comprises:

(a) providing a reagent capable of interacting with a sample suspected of containing a particular ligand species, which reagent comprises a labeled ligand or a labeled ligand receptor capable of forming a complex with said particular ligand species,

said label being capable of acting on an added substrate to produce a change in the concentration of a detectable species and which change is proportional to the amount of said particular ligand species in said sample;

(b) contacting said sample and said reagent with a microfabricated biosensor comprising (i) a base sensor sensitive to the concentration of said detectable species, (ii) a permselective layer, superimposed over at least a portion of said base sensor, having a composition and thickness sufficient to exclude substantially molecules with a molecular weight of about 120 or more while allowing the free permeation of molecules with a molecular weight of about 50 or less, and (iii) a receptor layer, superimposed over said base sensor and at least a portion of said permselective layer, comprising a sufficient amount of an immobilized ligand receptor capable of binding said labeled, particular ligand species or any complex thereof,

for a period of time sufficient to allow said immobilized ligand receptor to bind with said labeled ligand, particular ligand species, or any complex thereof;

(c) removing any material which remains unbound to said immobilized ligand receptor followed by the addition of said substrate;

(d) measuring the change in the concentration of said detectable species; and

(e) relating said change to the amount of said particular ligand species in said sample.

82. A method for assaying a sample for a particular ligand (analyte) species which comprises:

(a) providing a reagent capable of interacting with a sample suspected of containing a particular ligand species, which reagent comprises a labeled ligand receptor capable of forming a complex with said particular ligand species,

said label being capable of acting on an added substrate to produce a change in the concentration of a detectable species and which change is proportional to the amount of said particular ligand species in said sample;

(b) contacting a sample and said reagent with with a microfabricated biosensor comprising: (i) a base sensor sensitive to the concentration of said detectable species, (ii) a permselective layer, superimposed over at least a portion of said base sensor, having a composition and thickness sufficient to exclude substantially molecules with a molecular weight of about 120 or more while allowing the free permeation of molecules with a molecular weight of about 50 or less, and (iii) a receptor layer, superimposed over said base sensor and at least a portion of said permselective layer, comprising a sufficient amount of an immobilized ligand receptor capable of binding said particular ligand species of any complex thereof,

for a period of time sufficient to allow said immobilized ligand receptor to bind with said particular ligand species or any complex thereof;

(c) removing any material which remains unbound to said immobilized ligand receptor followed by the addition of said substrate;

(d) measuring the change in the concentration of said detectable species; and

(e) relating said change to the amount of said particular ligand species in said sample.

83. A method for assaying a sample for a particular ligand (analyte) species which comprises:

(a) providing a reagent capable of interacting with a sample suspected of containing a particular ligand species, which reagent comprises a labeled ligand capable of competing with said particular ligand species for available immobilized ligand receptors,

said label being capable of acting on an added substrate to produce a change in the concentration of a detectable species, which change is proportional to the amount of said particular ligand species in said sample;

(b) contacting said sample and said reagent with a microfabricated biosensor comprising (i) a base sensor sensitive to the concentration of said detectable species, (ii) a permselective layer, superimposed over at least a portion of said base sensor, having a composition and thickness sufficient to exclude substantially molecules with a molecular weight of about 120 or more while allowing the free permeation of molecules with a molecular weight of about 50 or less, and (iii) a receptor layer, superimposed over said base sensor and at least a portion of said permselective layer, comprising a sufficient amount of said immobilized ligand receptor capable of binding said labeled ligand or particular ligand species,

for a period of time sufficient to allow said immobilized ligand receptor to bind with said labeled ligand or particular ligand species;

(c) removing any material which remains unbound to said immobilized ligand receptor followed by the addition of said substrate;

(d) measuring the change in the concentration of said detectable species; and

(e) relating said change to the amount of said particular ligand species in said sample.

84. The method of claim 80, 81, 82 or 83 in which said microfabricated biosensor further comprises a photoresist layer, comprising a photoformable proteinaceous mixture, interposed between said permselective layer and said receptor layer.

85. The method of claim 80, 81, 82 or 83 in which said microfabricated biosensor further comprises an electrolyte layer interposed between said base sensor and said permselective layer.

86. The method of claim 80, 81, 82 or 83 in which said immobilized ligand receptor is selected from the group consisting of ionophores, cofactors, polypeptides, proteins, glycoproteins, enzymes, immunoglobulins, antibodies, antigens, lectins, neurochemical receptors, oligonucleotides, polynucleotides, molecules of DNA, molecules of RNA, active fragments or subunits or single strands of the preceding molecules.

87. The method of claim 80, 81, 82 or 83 in which said permselective layer is photodefined.

88. The method of claim 80, 81, 82 or 83 in which said particular ligand species is selected fromthe group consisting of ionophores, cofactors, polypeptides, proteins, glycoproteins, enzymes, immunoglobulins, antibodies, antigens, lectins, neurochemical receptors, oligonucleotides, molecules of DNA, molecules of RNA, viruses, organisms, fungi, fragments or subunits or single strands of the preceding entities.

89. The method of claim 82 which further comprises removing any material which remains unbound to said immobilized ligand receptor between steps (b) and (c).

90. The method of claim 81, 82 or 83 in which the operation of step (c) is accomplished by adding a substrate with the concommitant removal of any materials which remain unbound to said immobilized ligand receptor.

91. A method for assaying a sample for a particular antigenic species which comprises:

(a) providing a reagent capable of interacting with a sample suspected of containing a particular antigenic species, which reagent comprises a labeled antibody capable of forming a complex with said particular antigenic species, said label being capable of acting on an added substrate to produce a change in the concentration of a detectable species and which change is proportional to the amount of said particular antigenic species in said sample;

(b) contacting a sample and said reagent with a microfabricated biosensor comprising (i) a base sensor sensitive to the concentration of said detectable species, (ii) a permselective layer, superimposed over at least a portion of said base sensor, having a composition and thickness sufficient to exclude substantially molecules with a molecular weight of about 120 or more while allowing the free permeation of molecules with a molecular weight of about 50 or less, and (iii) a receptor layer, superimposed over said base sensor and at least a portion of said permselective layer, comprising a sufficient amount of an immobilized ligand receptor comprising an antibody capable of binding said particular antigenic species or any complex thereof,

(c) removing any material which remains unbound to said immobilized ligand receptor followed by the addition of said substrate;

(d) measuring the change in the concentration of said detectable species; and

(e) relating said change to the amount of said particular antigenic species in said sample.

92. A method for assaying a sample for a particular antigenic species, which method comprises:

(a) providing a reagent capable of interacting with a sample suspected of containing a particular antigenic species, which reagent comprises a labeled antigenic species, said label being capable of acting on an added substrate to produce a change in the concentration of a detectable species and which change is proportional to the amount of said particular antigenic species in said sampel;

(b) contacting said sample and said reagent with a microfabricated biosensor comprising (i) a base sensor sensitive to the concentration of said detectable species; (ii)a permselective layer, superimposed over at least a portion of said base sensor, having a composition and thickness sufficient to exclude substantially molecules with a molecular weight of about 120 or more while allowing the free permeation of molecules with a molecular weight of about 50 or less, and (iii) a receptor layer, superimposed over said base sensor and at least a portion of said permselective layer, comprising a sufficient amount of an immobilized ligand receptor comprising an antibody capable of binding said labeled antigenic species, particular antigenic species or any complex thereof,

for a period of time sufficient to allow said immoblized ligand receptor to bind with said labeled antigenic species, particular antigenic species or any complex thereof;

(c) removing any material which remains unbound to said immobilized ligand receptor to bind with said labeled antigenic species, particular antigenic species or any complex thereof;

(d) measuring the change in the concentration of said detectable species; and

(e) relating said change to the amount of said particular antigenic species in said sample.

93. A method for assaying a sample for a particular antibody which comprises:

(a) providing a reagent capable of interacting with a sample suspected of containing a particular antibody, which reagent comprises a labeled antigenic species or a labeled anti-antibody capable of forming a complex with said particular antibody,

said label being capable of acting on an added substrate to produce a change in the concentration of a detectable species and which change is proportional to the amount of said particular antibody in said sample;

(a) contacting a sample and said reagent with a microfabricated biosensor comprising (i) a base sensor sensitive to the concentration of said detectable species; (ii) a permselective layer, superimposed over at least a portion of said base sensor, having a composition and thickness sufficient to exclude substantially molecules with a molecular weight of about 120 or more while allowing the free permeation of molecules with a molecular weight of about 50 or less, and (iii) a receptor layer, superimposed over said base sensor and at least a portion of said permselective layer, comprising a sufficient amount of an immobilized ligand receptor comprising an antigenic species or anti-antibody capable of binding said particular antibody or any complex thereof,

for a period of time sufficient to allow said immobilized ligand receptor to bind with said particular antibody or any complex thereof;

(c) removing any material which remains unbound to said immobilized ligand receptor followed by the addition of said substrate;

(d) measuring the change in the concentration of said detectable species; and

(e) relating said change to the amount of said particular antibody in said sample.

94. A method for assaying a sample for a particular antibody, which method which comprises:

(a) providing a reagent capable of interacting with a sample suspected of containing a particular antibody, which reagent comprises a labeled antibody,

said label being capable of acting on an added substrate to produce a change in the concentration of a detectable species and which change is proportional to the amount of said particular antibody in said sample;

(b) contacting said sample and said reagent with a microfabricated biosensor comprising (i) a base sensor sensitive to the concentration of said detectable species, (ii) a permselective layer, superimposed over at least a portion of said base sensor, having a composition and thickness sufficient to exclude substantially molecules with a molecular weight of about 120 or more while allowing the free permeation of molecules with a molecular weight of about 50 or less, and (iii) a receptor layer, superimposed over said base sensor and at least a portion of said permselective layer, comprising a sufficient amount of an immobilized ligand receptor comprising an antigenic species or anti-antibody capable of binding said particular antibody or labeled antibody,

for a period of time sufficient to allow said immobilized ligand receptor to bind with said particular antibody or labeled antibody;

(c) removing any material which remains unbound to said immobilized ligand receptor followed by the addition of said substrate;

(d) measuring the change in the concentration of said detectable species; and

(e) relating said change to the amount of said particular antibody in said sample.

95. The method of claim 91, 92, 93 or 94 in which the operation of step (c) is accomplished by adding a substrate with the concommitant removal of any materials which remain unbound to said immobilized ligand receptor.

96. The method of claim 80, 81, 82, 83, 91, 92, 93 or 94 in which said label is an enzyme.

97. The method of claim 80, 81, 82, 83, 91, 92, 93 or 94 in which said detectable species is dioxygen or hydrogen peroxide.

98. The method of claim 80, 81, 82, 83, 91, 92, 93 or 194 in which said substrate is an indoxyl phosphate, analog, or derivative thereof.

99. A method for assaying a sample for a particular oligonucleotide sequence, which method comprises:

(a) providing a reagent capable of interacting with a sample suspected of containing a particular oligonucleotide sequence, which reagent comprises a labeled probe having a base sequence which is complementary to at least a portion of said oligonucleotide sequence and capable of forming a hybrid complex therewith,

said label being capable of acting on an added substrate to produce a change in the concentration of a detectable species and which change is proportional to the amount of said oligonucleotide sequence in said sample;

(b) contacting said sample and said reagent with a microfabricated biosensor comprising (i) a base sensor sensitive to the concentration of said detectable species, (ii) a permselective layer, superimposed over at least a portion of said base sensor, having a composition and thickness sufficient to exclude substantially molecules with a molecular weight of about 120 or more while allowing the

free permeation of molecules with a molecular weight of about 50 or less, and (iii) a receptor layer, superimposed over said base sensor and at least a portion of said permselective layer, comprising a sufficient amount of an immobilized ligand receptor comprising an antigenic species or anti-antibody capable of binding said oligonucleotide sequence or complex hybrid thereof,

for a period of time sufficient to allow said immobilized ligand receptor to bind with said oligonucleotide sequence or complex hybrid thereof;

(c) removing any material which remains unbound to said immobilized ligand receptor followed by the addition of said substrate;

(d) measuring the change in the concentration of said detectable species; and

(e) relating said change to the amount of said oligonucleotide sequence in said sample.

100. The method of claim 99 in which said immobilized ligand receptor is a preselected probe having a base sequence which is complementary to at least a portion of said oligonucleotide sequence and which binds said oligonucleotide sequence or hybrid complex thereof at a site other than that engaged by said labeled probe.

101. The method of claim 99 in which said immobilized ligand receptor is an antibody which recognizes said hybrid complex.

102. The method of claim 80, 81, 82, 83, 91, 92, 93, 94 or 99 in which said base sensor is an electrochemical sensor.

103. A method for assaying a sample for a particular analyte species which comprises:

(a) providing a reagent capable of interacting with a sample suspected of containing a particular analyte species, which reagent comprises a labeled analyte species or a labeled ligand receptor capable of forming a complex with said analyte species,

said label being capable of acting on an added substrate to produce a change in the concentration of an electroactive species and which change is proportional to the amount of said particular analyte species in said sample;

(b) contacting said sample and said reagent with a microfabricated device comprising (i) a base sensor comprising an electrochemical sensor sensitive to the concentration of said electroactive species, and (ii) an immobilized analyte receptor capable of binding said labeled analyte species, particular analyte species or complex thereof;

(c) removing any material which remains unbound to said immobilized analyte receptor followed by the addition of said substrate;

(d) measuring the change in the concentration of said electroactive species; and

(e) relating said change to the amount of said particular analyte species in said sample.

104. The method of claim 80, 81, 82, 83, 91, 92, 93, 94, 99 or 103 in which said base sensor is an electrochemical sensor comprising an amperometric electrode and a reference electrode.

105. A method for assaying a sample for a particular enzyme which comprises:

(a) providing a reagent capable of interacting with a particular enzyme suspected of being present in a given sample, which reagent comprises a substrate which undergoes a chemical transformation mediated by said

particular enzyme and which transformation gives rise to a change in the concentration of an electroactive species selected from the group consisting of dioxygen and hydrogen peroxide;

(b) contacting said sample and said reagent with a device comprising an electrochemical sensor sensitive to the concentration of said electroactive species;

(c) measuring the change in the concentration of said electroactive species; and

(d) relating said change to the amount of said particular enzyme in said sample.

106. The method of claim 105 in which said particular enzyme is a hydrolase and said reagent comprises an indoxyl moiety having a hydrolyzable functional group.

107. A method of establishing a dispensed layer onto a substantially planar surface comprising:

(a) preparing a fluid composition suitable for loading into a movable microsyringe assembly, said fluid composition having optimized surface tension and viscosity characteristics;

(b) loading said fluid composition into said movable microsyringe assembly, which assembly comprises (i) a reservoir for holding said fluid composition, (ii) a microsyringe needle, including an elongated member and a needle tip, (iii) means for delivering said fluid composition from said reservoir to said microsyringe needle, if said reservoir is displaced apart from said microsyringe needle, (iv) means for forcing controlled amounts of said fluid composition through said elongated member to form an emerging droplet of a predetermined volume on said needle tip, and (v) means for controlling the multidirectional movement of said assembly such that said droplet may be brought into contact with a preselected area of a substantially planar surface;

(c) optionally pretreating said surface under conditions sufficient to bring its surface free energy within a desired range;

(d) contacting said droplet on said needle tip with a preselected area of said surface; and

(e) retracting said assembly away from said surface such that said droplet disengages from said needle tip in a manner which provides a dispensed layer of said fluid composition having predictable and reproducible dimensions on said surface.

108. The method of claim 107 in which said substantially planar surface comprises a wafer having an array of unit cells of uniform dimensions.

109. The method of claim 108 in which said unit cells include a base sensor selected from the group consisting of amperometric and potentiometric sensors.

110. The method of claim 108 in which said unit cells include a base sensor selected from the group consisting of acoustic wave sensing devices, thermistors, gas-sensing electrodes, field-effect transistors, optical waver guides, evanescent field sensors, and conductimetric sensors.

111. The method of claim 107 in which said fluid composition includes a film-forming latex.

112. The method of claim 107 in which said fluid composition includes a photoformable proteinaceous mixture.

113. The method of claim 111 or 112 in which said fluid composition further includes one or more bioactive molecules.

114. The method of claim 107 in which said fluid composition includes a polymer matrix, a plasticizer, and an ionophore.